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Applicant: Brough

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DECLARATION UNDER 37 C.F.R. § 1.132 OF DOUGLAS E. BROUGH, PH.D.

1. I, Douglas E. Brough, am the inventor of the subject patent application. I currently am Executive Director, Head of Research at GenVec, Inc., which is the assignee of the subject patent application.

2. The following experiments were performed at my direction and under my supervision.

3. The ability of adenoviral vector-mediated gene delivery of an atonal-associated factor to change sensory perception was tested in a mouse model of vestibular (i.e., balance) dysfunction. Specifically, female C57BL/6 mice were treated with 120 μg/g body weight iminodipropionitrile (IDPN), which causes vestibular hair cell death resulting in vestibular dysfunction.

4. To assess vestibular function in IDPN-treated mice, the rotarod treadmill test was used. The rod started at an initial velocity of 4 rpm and accelerated to 40 rpm at 300 seconds. The time from the start of acceleration until the mouse fell completely off the rod was recorded.

The test was stopped after a maximum of 360 seconds. All mice were trained on the rod for two days prior to the baseline recording. The time that mice stayed on the rotating rotarod decreased significantly after exposure to IDPN. Immunohistochemistry of macular organs of treated mice revealed a severely damaged hair cell population in the saccule, utricle, and ampulla on day 7 after IDPN exposure, with the ampulla and utricle being more affected than the saccule. Rotarod times did not improve significantly for four weeks after IDPN exposure; however, rotarod times eventually improved and reached a plateau at 7 weeks.

5. Immunohistochemistry of inner ear hair cells on day 62 after IDPN exposure showed residual hair cells that looked healthier than on day 7 after IDPN exposure. However, the distribution of the residual hair cells was patchy, and the cells often lacked stereocilia. The results of the above-described experiment, therefore, demonstrate that IDPN produces a severe vestibular functional deficit in mice.

6. To test the impact of serotype 28 adenovirus (Ad28) on the vestibular system, healthy mice received an E1-deleted serotype 28 adenovirus vector comprising the green fluorescent protein (GFP) gene operably linked to a human CMV promoter (Ad28GFP) via the round window, which is one of the two openings into the cochlea of the inner ear. The preoperative baseline recording, i.e. the average time that animals managed to stay on the rotating rotarod, was 299 s ± 44.9 SD (n=4). The performance was stable with 311 s ± 35.6 SD on postoperative day 3, 309 s ± 33.5 SD on day 7, and 314 s ± 33.3 SD on day 14. There was no statistically significant change in the postoperative time period as compared to the preoperative time period ($p<0.05$).

7. Eleven days ("day 11") after the mice were exposed to IDPN, the mice were inoculated with an E1-deleted serotype 28 adenovirus vector comprising either (i) the atonal-associated factor 1 (atoh1) gene operably linked to a glial fibrillary acidic protein (GFAP) promoter (Ad28GFAP.atoh1) or (ii) the green fluorescent protein (GFP) gene operably linked to a human CMV promoter (Ad28GFP). Only one ear was treated so that the untreated ear could serve as a control. Specifically, 0.65×10^9 pu of Ad28GFAP.atoh1 or 0.14×10^8 pu of Ad28GFP was administered through the round window using a Hamilton micro syringe (Hamilton Company, USA).

8. After inoculation with Ad28GFAP.atoh1 on day 11, mice rotarod time improved. The recovery rate, measured in additional seconds on the rotarod per day, was not statistically significant for the Ad28GFAP.atoh1-treated animals during the first days after inoculation (6.77 s/d ± 8.5 SD for the period from day 10 to day 16) as compared to the IDPN-only group (3.63 s/d ± 4.99 SD for the period from day 10 to day 15). In the second week after vector delivery, the rotarod recovery rate for Ad28GFAP.atoh1-treated mice improved significantly faster (11.46 s/d ± 51.6 SD for the period from day 16 to day 24, P=0.047) than the IDPN-only controls (2.33 s/d ± 0.82 SD for the period from day 15 to day 28). However, after this period no further significant difference in the recovery rate was detectable between the two groups. The rotarod recovery rate from the time of vector administration at day 11 to sacrifice at day 62 was significantly better in Ad28GFAP.atoh1-treated mice (4.17 s/d ± 0.8 SD) than mice treated only with IDPN (2.01 s/d ± 0.96 SD, P=0.001). At the end of the experiment, Ad28GFAP.atoh1 treated-mice remained on the rotarod for 237 s ± 52.21 SD, which is 73.5 % ± 14.2 SD of the baseline performance before IDPN exposure. In contrast, the mice treated only with IDPN remained on the rotarod for 113 s ± 60.4 SD, which is 34.1% ± 16.2 SD of the baseline performance before IDPN exposure (P=0.005).

9. Macular organ cultures were immunostained for myosin VIIa, which is a selective marker for hair cells in the inner ear. In mice that received Ad28GFAP.atoh1 after exposure to IDPN, the total hair cell count significantly increased to 1668.3 ± 733.9 SD, as compared to mice treated only with IDPN (636.2 ± 462.3 SD). A statistically significant increase in hair cell numbers was observed in the sacculus (448.0 ± 207.3 SD, n=4) as well as in the utricle (1220.5 ± 619.6 SD, n=4) of Ad28GFAP.atoh1-treated mice. Statistical analysis revealed that there was no difference in hair cell numbers of Ad28GFAP.atoh1-treated mice as compared to healthy mice (P=0.384).

10. In mice receiving Ad28GFAP.atoh1 and IDPN, inner ear hair cells appeared healthy and exhibited well-defined myosin VIIa staining throughout the cell body and in the stereocilia. Some cell bodies were comparatively slender and reached into the bottom layer of the sensory epithelium, suggesting that they might have evolved from supporting cells through transdifferentiation.

11. Immunohistochemistry for neurofilaments (NFs) showed the presence of characteristic calyx afferents that ensheath type I vestibular hair cells. IDPN exposure

significantly reduced the level of NFs in the calyces, which is concordant with previous studies. In contrast, vestibular hair cells of mice that received Ad28GFAP.atoh1 after IDPN exposure exhibited well-defined calyces.

12. The results of the above-described experiments demonstrate that administration of a subgroup D adenoviral vector (i.e., Ad28) encoding an atonal-associated gene operably linked to a promoter that specifically functions in supporting cells of the inner ear (i.e., GFAP promoter) improves vestibular function in injured mice, most likely by inducing sensory hair cell growth in the inner ear.

13. The mere fact that an adenoviral vector is based on a non-subgroup C adenovirus, however, is not predictive of its ability to transduce cells of the inner ear. Indeed, while some non-subgroup C adenoviral vectors (e.g., Ad35 and Ad28) exhibit enhanced delivery to sensory cells of the inner ear as compared to a subgroup C adenoviral vector, other serotypes (e.g., serotypes 14 and 41 of subgroups B and F, respectively) transduce inner ear cells in a pattern similar to subgroup C vectors.

14. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 12/15/09

Douglas E. Brough, Ph.D.